

## Nematodes of the Indian Star Tortoise, *Geochelone elegans* (Testudinidae) with Description of a New Species *Alaeuris geochelone* sp. n. (Oxyurida: Pharyngodonidae)

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**ABSTRACT:** Five species of pharyngodonid nematodes were recovered from the large intestines of captive Indian star tortoises, *Geochelone elegans* (Schoepff, 1794). These were identified as *Alaeuris geochelone* sp. n., *Mehdiella microstoma*, *Tachygonetria conica nicollei*, *T. dentata quentini*, and *T. macrolaimus dessetae*. The characteristics of *A. geochelone* sp. n. are a very long esophagus, about half of the body, the hemicircular broad caudal alae that reach to the postanal caudal papillae, gubernaculum with rounded knob at distal end, and two claw-shaped lateral lips of the cloaca. All the nematodes found represent new host records.

**KEY WORDS:** pharyngodonid nematoda, Indian star tortoise, *Geochelone elegans*, *Alaeuris geochelone* sp. n., *Mehdiella microstoma*, *Tachygonetria conica nicollei*, *T. dentata quentini*, *T. macrolaimus dessetae*.

The Indian star tortoise, *Geochelone elegans* (Schoepff, 1794), Testudinidae, is an herbivorous reptile that is distributed in India, Pakistan, and Sri Lanka (Iverson, 1992). This is one of the tortoises listed under the Convention on the International Trade in Endangered Species of Wild Fauna and Flora (CITES). The tortoises studied in this work were provided to us from Maruyama Zoo, Sapporo City, Hokkaido, and Tennoji Zoo, Osaka City, Osaka, in Japan. They were seized by Customs at Nagoya, Japan, in November 1995 and were placed in zoos. Their origin is unknown.

The parasite fauna of *G. elegans* has not been previously studied, but 6 genera and about 54 species of Pharyngodonidae have been described from tortoises (Adamson, 1994). In the present study, we describe a new species of *Alaeuris* Thapar, 1925, and report 4 other species of Pharyngodonidae from the Indian star tortoise.

### Materials and Methods

Thirteen Indian star tortoises died in the Maruyama and Tennoji zoos between May and December 1996. Whole animals or viscera were fixed in 10% formalin. No specific cause of death was identified at necropsy, but nematodes were recovered from the large intestines of 7 tortoises. The nematodes were washed with water and cleared in lactophenol for identification. Drawings were made with an Olympus microscope drawing attachment. Measurements were done using an Olympus video micrometer (Model VM-30). Specimens for

scanning electron microscopy (SEM) were fixed in 2% buffer glutaraldehyde (pH 7.3). After being treated in 2% tannic acid, the specimens were washed in distilled water, postfixed in 1% osmium tetroxide, dehydrated in an ethanol series, and critical point dried. Specimens were coated with gold-palladium and examined with a Hitachi field emission scanning electron microscope (Model S-4100) at 15 kV. Measurements are in micrometers unless otherwise noted, with range followed by number measured and mean  $\pm$  standard deviation in parentheses.

### Results

Five species of nematodes were recovered, including a new species, *Alaeuris geochelone* sp. n. The other four species recovered are as follows: *Mehdiella microstoma* (Drasche, 1884) Seurat, 1918; *Tachygonetria conica nicollei* (Seurat, 1918) Petter, 1966; *Tachygonetria dentata quentini* Petter, 1966; and *Tachygonetria macrolaimus dessetae* Petter, 1966. Prevalence, mean intensity, and mean abundance of these species are presented in Table 1. All the nematodes found represent new host records. Specimens of each nematode species were deposited in the Laboratory of Parasitology, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Japan: *A. geochelone* sp. n. (Helm. Coll. No. 2973–2975), *M. microstoma* (Helm. Coll. No. 2976), *T. conica nicollei* (Helm. Coll. No. 2977), *T. dentata quentini* (Helm. Coll. No. 2978), and *T. macrolaimus dessetae* (Helm. Coll. No. 2979). The description of the new species follows.

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Table 1. Nematodes from large intestines of 13 captive Indian star tortoises.

Species	Prevalence	Mean intensity $\pm$ SD (range)	Mean abundance
<i>Alaeuris geocheilone</i> sp. n.	54% (7/13)	154.6 $\pm$ 217.35 (1–602)	83.2
<i>Mehdiella microstoma</i>	46% (6/13)	36.5 $\pm$ 40.19 (3–101)	16.9
<i>Tachygonetria conica nicolleti</i>	23% (3/13)	139.7 $\pm$ 146.37 (50–257)	32.2
<i>T. dentata quentini</i>	8% (1/13)	18.0 (18)	1.4
<i>T. macrolaimus dessetae</i>	38% (5/13)	97.0 $\pm$ 142.80 (2–349)	37.3

***Alaeuris geocheilone* sp. n.**  
(Figs. 1 and 2)

GENERAL: Pharyngodonidae Travassos, 1919, *Alaeuris* Thapar, 1925. Body small and whitish with thick cuticle. Oral opening triangular to circular surrounded by circum oral ridge. Two small amphids present. Four inner papillae observed in surface view in lactophenol-treated specimens. Six cuticular flaps projecting into buccal cavity. Esophagus elongate, about half of body length. Bulb well developed. Nerve ring at anterior part of esophagus, excretory pore at side of bulb.

MALE (holotype and 14 paratypes): Total length 2.11–2.71 ( $n = 15$ ,  $2.35 \pm 0.31$ ) mm. Maximum width 195–289 ( $234 \pm 42$ ) near middle of body. Six cuticular flaps projecting into buccal cavity (Fig. 2A). Nerve ring 151–185 ( $169 \pm 13$ ) and excretory pore 863–1,077 ( $943 \pm 72$ ) from cephalic extremity. Esophagus 1.01–1.27 ( $1.15 \pm 0.13$ ) mm in length including bulb. Bulb 101–122 ( $110 \pm 11$ ) long and 140–153 ( $146 \pm 6$ ) wide. Vas deferens broad and packed with sperm. Tail short 71–90 ( $78 \pm 7$ ) with hemicircular broad caudal alae. Caudal alae width 63–69 ( $66 \pm 3$ ). Three pairs of genital papillae present, preanal, adanal, and postanal. The caudal alae hemicircular, narrows ahead of posterior papillae. Cloaca surrounded by 2 claw-shaped lips. Spicule sharp and straight, 89–110 ( $100 \pm 9$ ) long. Gubernaculum Y-shaped, 31–45 ( $38 \pm 6$ ) long, terminating in knob bearing 2 small round projections (Fig. 2B).

FEMALE (allotype and 14 paratypes): Total length 2.70–3.65 ( $n = 15$ ,  $3.31 \pm 0.42$ ) mm. Maximum width 257–456 ( $345 \pm 92$ ) near middle of body. Three cuticular flaps projecting into buccal cavity. Nerve ring 180–245 ( $202 \pm 18$ ) and excretory pore 1.14–1.49 ( $1.29 \pm 0.13$ ) mm from cephalic extremity. Esophagus 1.50–2.05 ( $1.70 \pm 0.28$ ) mm in length including bulb. Bulb 125–141 ( $131 \pm 6$ ) long and 153–178 ( $162 \pm 10$ ) wide. Vulva 1.92–2.35 ( $2.22 \pm 0.26$ ) mm

from cephalic extremity. Tail conical, 113–167 ( $138 \pm 21$ ) long. Eggs unembryonated 109–134 ( $121 \pm 10$ ) long by 51–72 ( $60 \pm 8$ ) wide.

**Taxonomic summary**

TYPE HOST: Indian star tortoise, *Geochelone elegans* (Schoepff, 1794) (Testudines: Testudinidae).

SITE OF INFECTION: Large intestine.

TYPE LOCALITY: India, Pakistan, or Sri Lanka.

TYPE SPECIMENS: Holotype (Helm. Coll. No. 2973), allotype (Helm. Coll. No. 2974), and paratypes (Helm. Coll. No. 2975) deposited in Laboratory of Parasitology, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Japan.

ETYMOLOGY: The species was named after the host tortoise.

**Remarks**

Nematodes of the genus *Alaeuris* are parasites of herbivorous tortoises (Testudinidae) and lizards (Iguanidae) and rarely of carnivorous reptiles (Petter and Quentin, 1976). This genus was proposed by Thapar (1925) with type species *A. numidica numidica* (Seurat, 1918) Petter, 1966 (syn. *A. alaeuris* Thapar, 1925), from the large intestine of *Testudo graeca iberica* Pallas, 1814 (syn. *T. iberica*), and now includes 33 species (Baker, 1987). We placed the new nematode in the genus *Alaeuris*, because it possesses the robust caudal alae and the ventral papillae of the caudal appendage that are characteristic of *Alaeuris* (Petter and Quentin, 1976). We have found only 1 report on the occurrence of *Alaeuris* from *Geochelone radiata* (Shaw, 1802) (syn. *Testudo radiata*) in Madagascar (Petter, 1966). This species is *A. numidica madagascariensis*, but a number of species have been found from tortoises of the genus *Testudo*. The latter genus is closely related to *Geochelone*. The *Alaeuris* spp. recovered from *Testudo* are as follows: *A.*

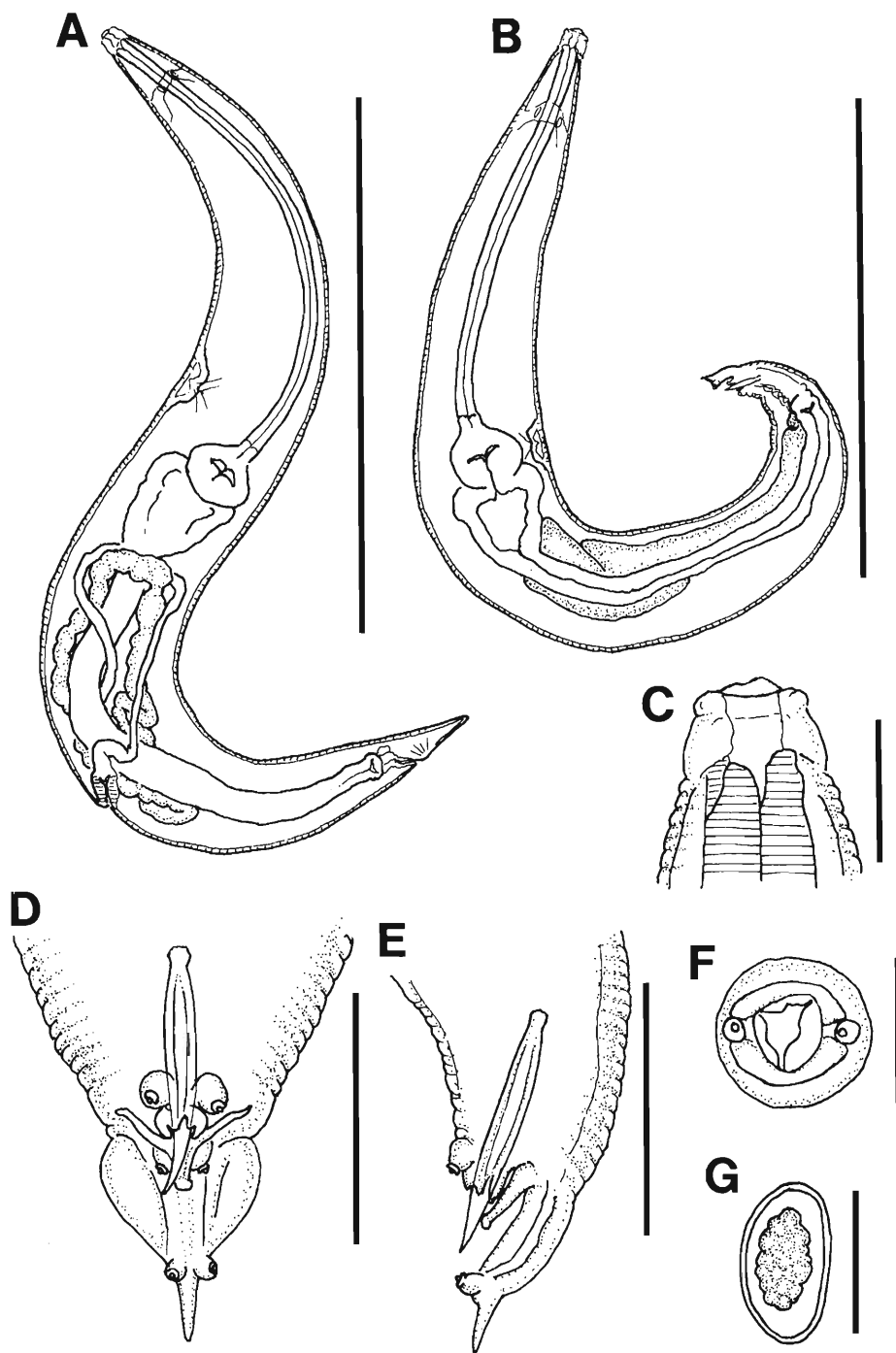


Figure 1. *Alaeuris geochelone* sp. n. A. Female. B. Male. C. Cephalic end of female, lateral view. D. Caudal end of male, ventral view. E. Caudal end of male, lateral view. F. Cephalic end of female, apical views. G. Egg. Scale bar: A, B = 1 mm; C = 50  $\mu$ m; D, E = 100  $\mu$ m; F = 50  $\mu$ m; G = 100  $\mu$ m.

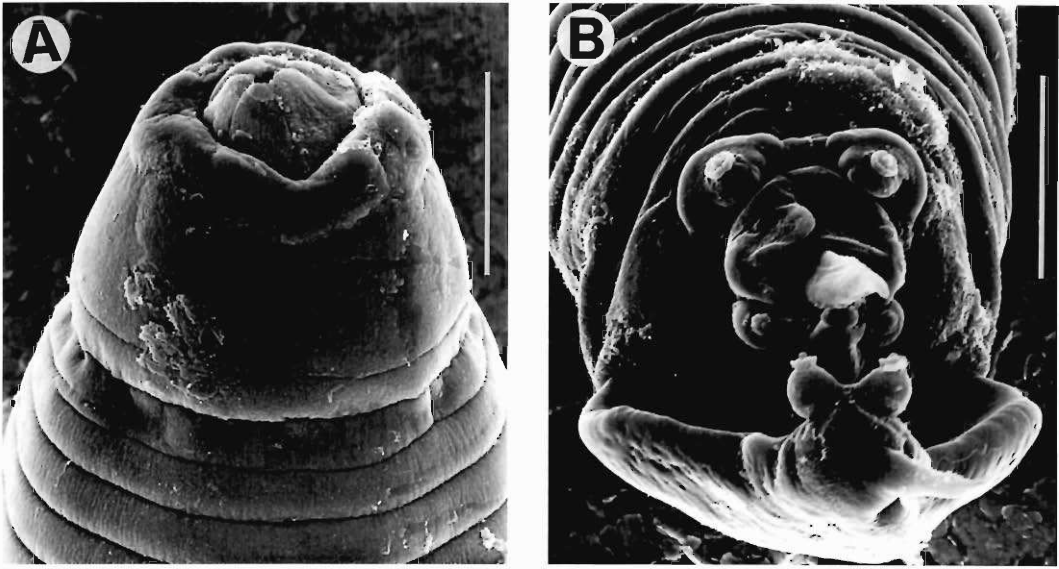


Figure 2. *Alaeuris geocheilone* sp. n., SEM micrographs. A. Cephalic end of male, lateral view. B. Caudal end of male. Bar = 20  $\mu$ m.

*auricularis* (Walton, 1942) Petter, 1966, *A. dupuisi* Petter, 1966, *A. macroptera* (Walton, 1942) Petter, 1966, *A. numidica numidica* (Seurat, 1918) Petter, 1966, *A. pharyngodentata* (Walton, 1942) Petter, 1966, *A. quadrilabiata quadrilabiata* Ortlepp, 1933, and *A. quadrilabiata insularis* Petter, 1966. Morphologically, the new nematode is most similar to *A. n. numidica* and *A. n. madagascariensis*, but it can be easily distinguished by characters such as a very long esophagus about half of the body length, the hemicircular broad caudal alae that reach the postanal caudal papillae, gubernaculum with

rounded knob at the distal end, and 2 claw-shaped lateral lips of the cloaca. Body size of *A. geocheilone* sp. n. is smaller than *A. n. numidica* and *A. n. madagascariensis*. Measurements of these nematodes are presented in Table 2. Petter and Brygoo (1972) reported body size variation of nematodes by host species; however, our new species has other distinct characteristics. The spicule (100  $\mu$ m) is shorter than *A. n. numidica* (180  $\mu$ m) but is not as short as *A. n. madagascariensis* (80  $\mu$ m). Petter (1966) described the cuticle of *A. n. madagascariensis* as detached from the body in the posterior part, but this char-

Table 2. Comparison of *Alaeuris numidica* and *A. geocheilone* sp. n.

	Petter, 1966 <i>Alaeuris numidica numidica</i>		Petter, 1966 <i>A. n. madagascariensis</i>		Our specimens <i>A. geocheilone</i> sp. n.	
	Female	Male	Female	Male	Female (n = 15)	Male (n = 15)
Total length (mm)	5.7	3.4–4.7	5.2	3.8	3.31 $\pm$ 0.42	2.35 $\pm$ 0.31
Maximum width	400	260–300	500	300	345 $\pm$ 92	234 $\pm$ 42
Nerve ring	300	200	250	200	202 $\pm$ 18	169 $\pm$ 13
Excretory pore (mm)	2.2	1–1.8	1.18	1.4	1.29 $\pm$ 0.13	943 $\pm$ 72
Vulva from cephalic extremity (mm)	3.9		3.5		2.22 $\pm$ 0.26	
Esophagus (mm)	2.25	1.55–1.65	1.65	1.35	1.70 $\pm$ 0.28	1.15 $\pm$ 0.13
Tail	150	80–90	150	60	138 $\pm$ 21	78 $\pm$ 7
Eggs	120 $\times$ 70		140 $\times$ 60		121 $\pm$ 10 $\times$ 60 $\pm$ 8	
Spicule		180		80		100 $\pm$ 9

acter was not observed in our specimens. This is the first report of *Alaeuris* from *G. elegans*.

### Discussion

Indian star tortoises in this study were classified in the genus *Geochelone* according to the taxonomy of Ernst and Barbour (1989). Unfortunately, their origin is unknown; however, they may have originated from India, Pakistan, and Sri Lanka, where they normally occur (Iverson, 1992).

Members of family Testudinidae are terrestrial tortoises and are found primarily in tropical portions of Africa, Madagascar, India, Southeast Asia, South America, Aldabra Atoll, and the Galapagos Archipelago (Ernst and Barbour, 1989). However, the fossil record shows tortoises were much more widespread in northern Europe and England, central Asia, the West Indies, and North America to southern Canada. The family is presently composed of 12 genera and 50 species (Ernst and Barbour, 1989).

The first reptile appeared in the Triassic. Parasitic nematodes of vertebrates are presumed to be derived from soil nematodes (Bain and Chabaud, 1979), and parasitism probably began with the appearance of terrestrial vertebrates (Anderson, 1984). According to Baker (1984), most nematode groups that evolved in amphibians and reptiles have been shown to be at least Mesozoic in age. Older amphibian and reptilian groups have proportionally more nematode species than the more recently evolved groups (Baker, 1984).

Two or more species of Oxyurida frequently occur in the same host individual (Petter, 1966). We observed concurrent infections of 5 species in 1 host in this study. *Alaeuris geochelone* sp. n. had the highest intensity and prevalence among the nematodes collected (Table 1). All nematodes are the first record from *G. elegans* from South Asia. The same nematodes have been reported from Europe, Africa, and Russia (Baker, 1987). It would appear, therefore, that these parasitic nematodes evolved in tortoises before their hosts dispersed.

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